



Methane production enhancement and comparative study of biodegradation of some plants and animal wastes

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This study investigated the production of methane using various substrates. Plantain peels, pig dung, poultry droppings, walnut peels/plantain peels and cow blood/wheat waste were all co-digested, charged in digesters (A-E) and allowed to ferment anaerobically for forty-five (45) days within the mesophilic temperature range of 20.0-39.0°. Digester A contained 3.9 Kg of wheat waste/cow blood and 19.5 Kg of water in the ratio of 1.5 which gave a total gas yield of 22.5 L of biogas; B contained 7.8 Kg of walnut peels/plantain peels and 15.6 Kg of water in the ratio 1:2 which gave a total gas yield of 115.0 L; C contained 8 Kg of plantain peels and 16 Kg of water which yielded 133.0 L of biogas; D contained 8 Kg of pig dung mixed thoroughly with 16 Kg of water which gave a total of 321.0 L; and E contained 8 Kg of poultry droppings mixed with 16 Kg of water which yielded 168.5 L of biogas. From the cumulative comparison of the biogas yields of the samples, pig dung gave the highest yield of 321 L and cow blood/wheat husk produced the lowest yield of 22.5 L. Pig dung (animal waste) gave the highest total viable count (TVC) of 7×10^6 cfu/mL. The sludge contains NPK which is a good biofertilizer.

Keywords: Anaerobically, Cumulative comparison, Digesters, Mesophilic, Substrate

It is necessary to focus on basic and applied research in order to find the best ways and solutions to energy scarcity so as to promote the valorization of the generated waste from a huge number of sources either by manufacturing new products with high added value or producing energy¹. Increasing population levels, a booming economy, rapid urbanization and the rise in community living standards have greatly accelerated the municipal solid waste generation rate in developing countries². The production of fruits and vegetable waste is also very high and is becoming source of concern in municipal landfills because of their high biodegradability³. The synthesis of biomass to produce energy is a growing trend worldwide as the quest for clean energy alternatives instead of the traditional fossil fuels intensifies⁴. Growing energy demand and the impacts of fossil fuel contribute towards the commercialization of biogas as a finite energy source. Organic waste materials are the major attraction for biogas production; even as biogas production through anaerobic digestion ensures alternative fuel, biofertilizer, electricity production,

waste recycling, greenhouse gas reduction, and environmental protection^{5,41}. Millions of homes in less-developed regions, including China and parts of Africa, are estimated to use household digesters as a renewable energy source³⁵.

Methane is the combustible fraction of biogas whereas relatively lower methane content of typical biogas in contrast to conventional energy sources is an added drawback that restrains the acceptance of biogas for commercial purposes⁴. However, ordinary waste materials or feedstock could not provide sufficient biogas⁴. The major aspect for sustainable feedstock utilization is united with the characteristics of the substrate. Substrate availability is another concern that has to be assured for commercialization of biogas in future³. In spite of this criterion animal manure was the most frequently used feedstock than agro industrial wastes until recent past. On the other hand, it may be due to the fact that manures possess low lignocelluloses content and can be effortlessly degradable by the microorganisms inhabited with it⁴. The accessibility of complex organic materials is a difficult task that requires further treatments to ensure absolute degradation and thereby biogas production⁶.

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The world is unavoidably faced with crises of fossil fuel shortage and environmental degradation as a result of growth in population, urbanization and industrialization⁷. Biogas is a mixture of gases evolved from the digestion process of organic matter by anaerobic bacteria at anaerobic conditions and many factors have been found that can control the efficiency of biogas production^{8,38}. Most studies about biogas indicate that methane (CH₄) and carbon dioxide (CO₂) are the main components, where the ratio of methane ranged between 50 - 80% and the ratio of carbon dioxide range is 20 - 50%⁴². The carbon dioxide fraction reduces the calorific value of biogas⁹. Other components of biogas that may be found in small amounts (traces) are Hydrogen (H₂), Nitrogen (N₂), Hydrogen Sulfide (H₂S), Carbon monoxide (CO), Ammonia (NH₃), Oxygen (O₂) and water vapor (H₂O). Methane and carbon dioxide are odorless and colorless gases. Hydrogen sulfide is colorless but it has an odor of rotten eggs in addition to its toxicity⁹.

Anaerobic bioreactors (ABs) have potential applications for rapid digestion of solid organic waste constituents to reduce the environmental load as compared to conventional sanitary landfills^{10,11}. Bioreactor design has been found to exert a strong influence on the performance of a digester¹². A variety of new bioreactor designs have been developed in recent years which facilitate a significantly higher rate of reaction for the treatment of waste¹³⁻¹⁵. According to Chandrasekhar and his co-workers, an anaerobic bioreactor should be designed in a way that allows a continuously high and sustainable organic load rate with short hydraulic retention time and has the ability to produce the maximum level of methane¹⁶. Anaerobic digestion (biogas technology) of organic wastes produces both biofertilizer and biogas³⁷. Unlike composting, the digestion process retains and even improves the nutrient value of the original feed stock¹⁷. Many methods and technologies could be applied to treat organic wastes such as direct combustion,

fermentation, gasification, pyrolysis, and anaerobic digestion^{18,40}. The aim of the present study was to generate and compare the physicochemical characteristics of biogas produced by co-digesting walnut waste and plantain peels, wheat husk, and cow blood substrate mixture.

Materials and Methods

The materials used for this study include five fixed dome biodigesters of volume 30254.72 cm³ (≈30 L), wheat husk (waste) and cow blood, pre-decayed walnut peels and plantain peels, pig dung; poultry droppings, weighing balance, pH paper, and electronic pH meter, plastic bowl and a 20 L gallon; gas hose, bamboo stick, and water. The batch operation method was used in this study.

Preparation of the samples

Solid and granular wastes like the walnut and plantain peels were first ground by mashing until a soft pulpy texture was obtained. The wastes were then mixed with water at specific ratios and fed separately into the digesters (A-E). Cow blood and wheat waste were mixed together and the combination mixed with water at a ratio of 1:5 and fed into digester A. Walnut and plantain peels were mixed with water at a ratio of 1:2 and fed into digester B. Plantain peels charged (independently) were mixed with water at a ratio of 1:2 and fed into digester C. Pig dung was mixed with water at a ratio of 1:2 and fed into digester D. Poultry droppings with water at a ratio of 1:2 were fed into digester E (Table 1).

Experiments A, B, C, D, and E were monitored for a period of 45 days until the biogas production rate became low. The records of volume and temperature were taken accordingly. A mercury-in-glass thermometer calibrated in centigrade from -10 to 110°C was used to measure both ambient and slurry temperatures. The volume of gas produced was obtained using the method of water displacement. Measurement of volume for each experiment was taken

Table 1 — Mixing ratio and the temperature of the samples

Wastes	Mixing ratio	Quantity of waste/water (Kg)	Ambient temp. range (°C)	Slurry temp. range (°C)	Volume of gas produced (L)
Pig dung(pd)	1:2	8:16	21.5-31.5	24.0-37.0	321.0
Poultry dropping(pod)	1:2	8:16	21.5-32.0	23.0-36.5	168.5
Plantain peel(pp)	1:2	8:16	21.5-31.5	24.0-38.8	131.0
Walnut/plantain(w/p)	1:2	7.8:15.6	22.0-34.5	24.0-38.8	115.0
Cow blood/wheat(b/w)	1:5	3.9:19.5	21.5-31.5	24.0-38.0	22.5

on a daily basis. For the measurement of pH, an electronic pH meter at the laboratory of the National Centre for Energy Research and Development was employed alongside a pH paper (for rough determination). Measurement was taken on a weekly basis. A Bunsen burner and a matchbox were used for flammability test. Flammable gas burned with a blue flame while non-flammable gas simply did not burn. The flammability was observed as shown (Table 2).

Qualitative analyses of the wastes

Proximate analysis for each of the samples was carried out in the laboratory section of the National Center for Energy Research, and development, University of Nigeria. Since the initial and final (0th & 45th day) samples from the digesters were collected and then evaluated for total solids, volatile solids,

carbon, nitrogen, lignin, and cellulose content^{5,10}. The common parameters such as pH and temperature of fresh substrate mixture and digested slurry were examined through the digital pH meter and thermometer. The total viable count determinations for microbes in each sample were carried out in the pharmaceutical laboratory of the University of Nigeria, Nsukka³⁹ (Tables 3-9).

Characteristics of the biogas produced

Each sample of biogas produced was analyzed using Orsat Apparatus. The measuring principle of Orsat Apparatus is the measurement of the reduction which occurs when individual constituents of gas are removed separately by absorption in liquid reagents (Table 10).

A gas compressor is a mechanical device that increases the pressure of a gas by reducing its volume.

Table 2 — Retention time and days of flammability

Wastes	Retention time(days)	Flammable time (days)	Volume of gas produced(L)
Pig dung(pd)	45	8	321.0
Poultry dropping(pod)	45	9	168.5
Plantain peel(pp)	45	6	131.0
Walnut/plantain(w/p)	45	12	115.0
Cow blood/wheat(b/w)	45	-	22.5

Table3 — Proximate Analysis for Pig dung and Poultry dropping

Parameter	Pig dung		Poultry dropping	
	Before digestion (%)	After digestion (%)	Before digestion (%)	After digestion (%)
Nitrogen	0.61	3.06	1.99	2.25
carbon content	1.88	1.63	14.12	8.27
pH	6.00	7.61	8.10	8.60
Ash	2.35	6.00	4.90	1.90
moisture	88.46	78.20	69.80	87.40
phosphorus	0.08	0.54	0.31	0.90
Volatile solid	71.65	69.35	81.11	80.35
Total solid	96.45	78.20	87.40	70.79
potassium	0.65	0.76	0.43	0.6

Table 4 — Proximate analyses of walnut/plantain and plantain peels

Parameter	Walnut/plantain (w/p)		Plantain peel (pp)	
	Before digestion (%)	After digestion (%)	Before digestion (%)	After digestion (%)
Nitrogen	0.13	0.27	0.07	0.17
carbon content	6.98	4.92	4.83	2.50
pH	8.33	7.20	9.48	9.00
Ash	0.79	0.38	0.86	0.60
moisture	94.00	96.36	94.34	96.55
Volatile solid	1.00	0.42	0.71	0.27
Total solid	1.28	0.60	0.92	0.40
fiber	2.83	1.35	3.07	1.79
protein	1.67	0.78	1.05	0.44
Fat	0.04	0.03	0.04	0.01

Table 5 — Characteristics of the five wastes used during the experimental study

Wastes	Total solid (%)	Volatile solid (%)	Total carbon (%)	Total nitrogen (%)	C/N ratio
Pig dung (pd)	78.20	69.35	10.63	3.06	3.47
Poultry dropping (pod)	70.79	80.35	14.12	1.99	7.09
Plantain peel (pp)	0.92	0.71	4.83	0.17	2.84
Walnut/plantain (w/p)	1.28	1.00	6.98	0.27	2.59
Cow blood/wheat (b/w)	0.92	0.75	5.10	0.17	3.80

Table 6 — Total viable count determination for bacteria

Wastes	Mean drop count	Dilution factor	Vol./drop (mL)	Total viable count (cfu/mL)
Pig dung (pd)	11	10 ²	0.015	7 × 10 ⁶
Poultry dropping (pod)	15	10 ²	0.015	1 × 10 ⁷
Plantain peel (pp)	11	10 ⁻⁴	0.02	5.5 × 10 ⁶
Walnut/plantain (w/p)	5	10 ⁻⁴	0.02	2.5 × 10 ⁶
Cow blood/wheat (b/w)	9	10 ⁻⁴	0.02	4.5 × 10 ⁶

Table 7 — Total viable count determination for fungi

Wastes	Mean drop count	Dilution factor	Vol./drop (mL)	Total viable count (cfu/mL)
Pig dung (pd)	None	None	None	None
Poultry dropping (pod)	none	None	None	None
Plantain peel (pp)	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>
Walnut/plantain (w/p)	none	None	None	None
Cow blood/wheat (b/w)	none	None	None	None

Table 8 — Organism isolated from the three samples

Wastes	Total viable count (cfu/mL)	Grams character	Organism isolated
Cow blood/wheat (b/w)	4.5	Positive	<i>Sarcinaitea</i>
		Negative	<i>Salmonella spp</i>
		Positive	<i>Bacillus cerus</i>
		Negative	<i>E. coli</i>
Walnut/plantain (w/p)	2.5	Negative	<i>E.coli</i>
		Positive	<i>Bacillus subtilis</i>
		Negative	<i>Salmonella spp</i>
		Positive	<i>Bacillus subtilis</i>
Plantain peel (pp)	5.5	Positive	<i>Bacillus subtilis</i>
		Negative	<i>E. coli</i>
		-	<i>Aspergillus niger</i>
		Negative	<i>Salmonella s[pp</i>

The capacity of the compressor used was 1/5 horse-power. Each cylinder was able to compress biogas to 1.2 bars of pressure.

Results and Discussion

The daily ambient temperature and slurry temperature for the five different wastes were shown in (Figs. 1A & B) while the daily volume of the gas produced versus retention time (Fig. 2). Walnut husk

with Plantain peels recorded the highest temperature range of 22.0-34.5°C and the five wastes produced biogas within the mesophilic range of temperature⁹ (Evans, 2016), walnut/plantain (w/p) and Plantain peels (pp) recorded the highest slurry temperature range of 24.0 - 38.8°C. These ranges of temperature favour the mesophilic bacteria and all the samples in the digesters were agitated twice per day to achieve degradation.

Table 9 — Summary of the organism(s) isolated from the three samples

Samples	Organism isolated	Type of organism	Gram characteristics
A ₁	<i>Sarcinaintea</i>	Bacteria	+ve
A ₂	<i>Salmonella spp</i>	Bacteria	-ve
A ₃	<i>Bacillus cerus</i>	Bacteria	+ve
A ₄	<i>E. coli</i>	Bacteria	-ve
B ₁	<i>E. coli</i>	Bacteria	-ve
B ₂	<i>Bacillus subtilis</i>	Bacteria	+ve
B ₃	<i>Salmonella spp</i>	Bacteria	-ve
C ₁	<i>Bacillus subtilis</i>	Bacteria	+ve
C ₂	<i>E. coli</i>	Bacteria	-ve
C ₃	<i>Aspergillus niger</i>	Fungi	-ve
C ₄	<i>Salmonella spp</i>	Bacteria	-ve

NB: A, B, C = Experiment A B C

Table 10 — Percentage of the component of biogas from five different wastes Using Orsat Apparatus

Wastes	Carbon dioxide (CO ₂) (%)	Hydrogen sulphide H ₂ S (%)	Carbon monoxide (CO) (%)	Methane and other components (%)
Pig dung (pd)	24.9	1.2	3.7	70.2
Poultry dropping (pod)	17.4	0.3	0.5	81.9
Plantain peel (pp)	19.5	0.8	9.0	70.7
Walnut/plantain (w/p)	16.5	0.9	0.3	82.3
Cow blood/wheat (b/w)	-	-	-	-

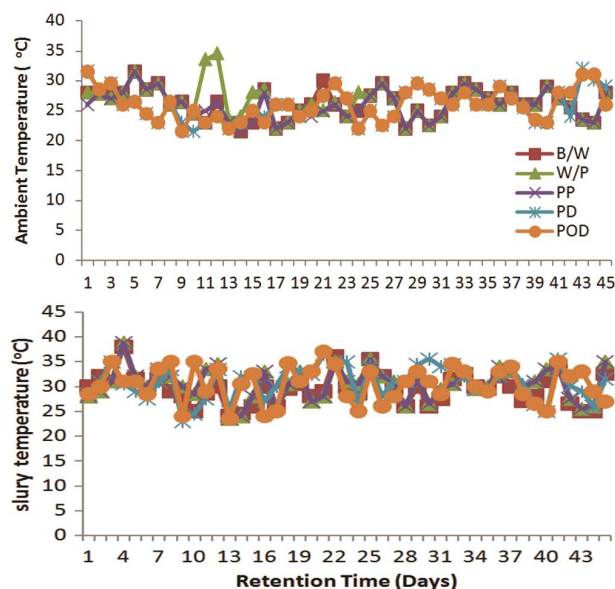


Fig. 1 — Change in (A) Ambient Temperature; and (B) Slurry temperature during Fermentation

The daily volumes of biogas yield of the five different wastes were shown in (Fig. 2). The curves show that the pig dung generated the highest gas from the first day to the 45th day. It was followed by poultry droppings which produced the highest between 20th and 43th days. Cow blood/wheat husk produced the highest gas on the 2nd day of digestion.

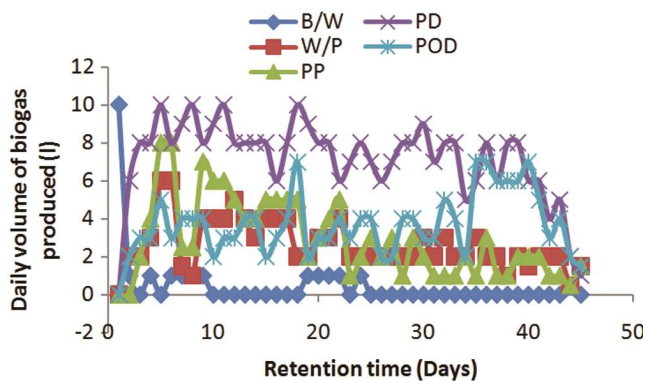


Fig. 2 — Volume of gas produced by five wastes during fermentation

On the 3rd to 10th days and the 20th to 25th days it produces about 1.5 L of gas. On the other days, it did not produce any gas and its gas never became combustible, it was discovered that cow blood is not a good inoculum and it contains lignin⁵. Walnut husk with Plantain peels produced the highest gas on the 8th day and became combustible on the 12th day. The biogas produced burned with a blue flame and has the highest calorific value. Co-digesting walnut and plantain gave low gas yield than the independently charged plantain peels. Apart from Cow blood/wheat husk which did no yield biogas within the period of study, and the order of gas yield was pig dung>poultry droppings>Walnut/plantain>Plantain peels.

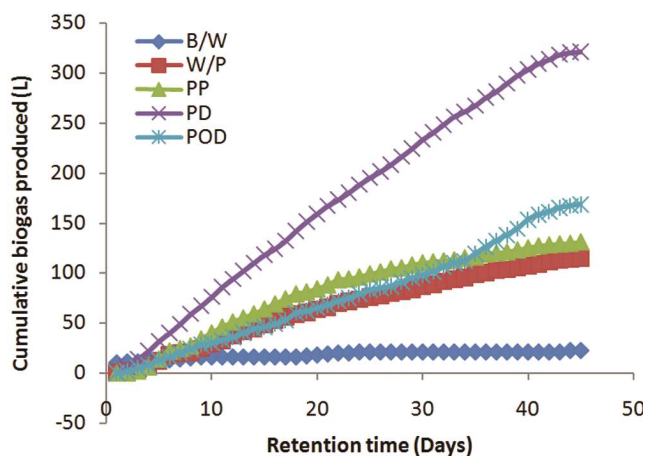


Fig. 3 — Cumulative gas produced by five different wastes

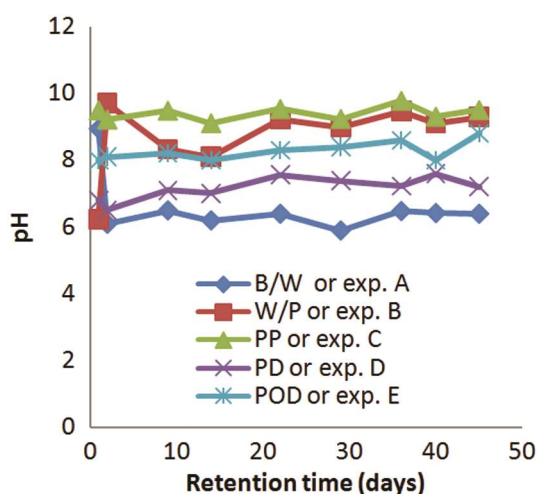


Fig. 4 — Change in pH during Fermentation

On the other hand, the pig dung slurry produced combustible gas on the 8th day and Plantain peels on the 6th day (Table 2). The cumulative biogas yields of the sample are compared in (Fig. 3). The pig dung gave the highest yield of 321 L and Cow blood/wheat husk produced the lowest yield of 22.5 L. From (Table 3) and 4 the pH values of the four samples range from 6 to 9. Pig dung and poultry droppings produced a combustible gas at the pH range of 7.71 and 8.60. While walnut/plantain and plantain peels produced a combustible gas at the pH range of 7.20 and 9.00.

For optimum functioning, the anaerobic microorganisms require a neutral environment. Figure 4 shows the change in pH during fermentation. A range of pH values suitable for anaerobic digestion has been reported by various researchers, but the optimal pH for methanogenesis has been found to be around 7.0. It was showed that the most favorable range of pH to

attain maximal biogas yield in anaerobic digestion is 6.5–7.5. The moisture content of all the samples ranges from 69.80% to 96.55%. High moisture contents usually facilitate the anaerobic digestion; however, it is difficult to maintain the same availability of water throughout the digestion cycle. It has been reported by^{19, 20} that the highest methane production rates occur at 60–80%. They found that the onset of the methanogenic phase took place around day 70 in both cases, at 70% and 80% moisture. The carbon/nitrogen (C/N) ratio of the samples used in this study runs from 2.59 to 7.09.

The rate of anaerobic digestion is strongly affected by the type, availability, and complexity of the substrate which is in line with the research^{21,22}. Different types of carbon sources support different groups of microbes⁹. Before starting a digestion process, the substrate must be characterized for carbohydrate, lipid, protein, and fiber contents²³. In addition, the substrate should also be characterized by the quantity of methane that can potentially be produced under anaerobic conditions. Carbohydrates are considered the most important organic component of municipal solid waste for biogas production⁹. Nitrogen is essential for protein synthesis and primarily required as a nutrient by the microorganisms in anaerobic digestion²⁴. Nitrogenous compounds in the organic waste are usually proteins which are converted to ammonium by anaerobic digestion²⁵. In the form of ammonium, nitrogen contributes to the stabilization of the pH value in the bioreactor where the process is taking place. Microorganisms assimilate ammonium for the production of new cell mass. The C/N ratio in the organic material plays a crucial role in anaerobic digestion.

The unbalanced nutrients are regarded as an important factor limiting anaerobic digestion of organic wastes. For the improvement of nutrition and C/N ratios, the co-digestion of organic mixtures is employed²⁶. Both acid and methane forming bacteria could not survive the pH values of 4 and 10. The different bacteria and fungi that aided degradation of substrate were listed in (Tables 6-9). The total viable count determination for bacteria of the five samples runs from 7×10^6 cfu/mL to 1×10^7 cfu/mL. The anaerobic digestion process can be catalyzed by a variety of microorganisms that convert complex macromolecules into low molecular weight compounds. An inoculum source is crucial for the optimization of the waste/inoculum ratio^{27,28}. Wet (40-95%) organic materials with low lignin and cellulose content are

generally suitable for anaerobic digestion³⁷. A wide variety of microbial communities have been reported to be involved in the anaerobic decomposition process²⁹ reported that organic material is most likely decomposed by heterotrophic microorganisms while³⁰ reported that *Clostridium* species are most common among the degraders under anaerobic condition.

Different wastes were used in feeding the digester in order to find out which one produced more biogas. It was found that organic waste which is easily digestible produced more gas. Based on recent research materials, high lignocellulose produces less amount of gas²⁰. Carbon, which constitutes the basic frame of all organic substrates provides energy used by the microbes for their living activities and is the source for the formation of biogas. In biogas production, nitrogen provides methanogenic bacteria with ammonia, which is the source of nitrogen for the composition of living matter of new cells³³. The carbon/nitrogen ratios for all the wastes were listed in (Table 6). The ideal carbon to nitrogen ratio for anaerobic digestion is between 20:1 and 30:1 according to studies of Ogbene, Andrew & Sunday³⁶.

The slurry should not be too thick nor too dilute. In this experiment, the dilution ratios used for all the wastes were recorded in (Table 1). Enough microbes were present in the digester to fasten the fermentation process. This was shown in the total viable count determination in (Tables 6 & 7). Gas production was found to be low at pH 4 and 9. The reason for the low pH values at the beginning of digestion was attributed to the fact that initially the acid-forming bacteria will be breaking down the organic matter and producing volatile fatty acids. As a result, the general acidity of the digesting material will increase and the pH will fall below neutral this is in agreement with work done by Lee *et al.*³¹. It was after week 2 of fermentation that the acid formers were most probably replaced by the methane forming bacteria and there is a gradual rise in the pH to 7.40 as shown in (Fig. 4).

Meanwhile, anaerobic degradation efficiency strictly depends on the characteristics of the samples used for charging the digester. Stirring also is necessary for increased gas production. When the slurry was stirred once in a day, there was an increase in gas production. There was also a drop in gas production when stirring was completely omitted due to scum formation. After the analysis of the slurry, it was discovered that there was an increase in the percentage content of nitrogen, potassium, protein,

and phosphorus after digestion. This shows that the sludge is a better fertilizer to the soil (Table 3)^{32,33}. The sludge is a potential organic manure³⁴.

Table 2 shows that plantain peels gave the shortest flammability time of 6 days followed by pig dung (8 days), poultry droppings (9 days), walnut husk/plantain peels (12 days), while cow blood/wheat chaff was not flammable within the period of study. The gas analyser and Orsat apparatus were used for the analysis of the composition of biogas produced (Table 10). The methane content of the biogas produced was in this order: the highest - walnut husk/plantain peels, next, poultry droppings, then plantain peels, and the least, pig dung.

Conclusion

The study revealed that animal wastes (pig dung and poultry dropping) anaerobically digested have higher yields of biogas in comparison with plant wastes (plantain peels and walnut husk/plantain peels). On the other hand, walnut husk/plantain peels yielded biogas faster than pig dung while pig dung produced a larger amount of biogas. Cow blood/wheat husk does not have enough carbon and nitrogen elements that meet the C/N ratio for optimum biogas yield. It was further observed that pig dung and poultry droppings were found to be better bio-fertilizer because of the presence of a higher volume of NPK in the digested wastes compared with those of walnut husk/plantain peels. Furthermore, walnut husk/plantain peels showed the highest yield for methane production, meanwhile, *Aspergillus nigers* were the only microbial (fungi) isolated in plantain peels while *Garcinia tea*, *Salmonella* spp, *E. coli*, and *Bacillus cereus* were the microorganisms associated with fermentation of the wastes.

Conflict of interest

All authors declare no conflict of interest.

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